

Preliminary Amendment

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Applicant(s): Michael S. KINCH et al.

Serial No.: 09/640,952

Filed: August 17, 2000

For: EPHA2 AS A DIAGNOSTIC TARGET FOR METASTATIC CANCER (As Amended)

Q12

16. (Amended) The method of claim 14 wherein the tyrosine phosphorylated proteins [are] comprise EphA2.

Please add the following new claims:

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28. A method for detecting the presence of metastatic or potentially metastatic cells in a cell population comprising:

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- lysing at least a portion of the cell population;
incubating the lysed cells with a reagent capable of specific binding to EphA2 to allow binding of the reagent to EphA2; and
detecting reagent-EphA2 binding, wherein reagent-EphA2 binding is indicative of the presence of metastatic or potentially metastatic cells in the cell population.

29. The method of claim 28 wherein the reagent comprises an antibody and wherein detecting reagent-EphA2 binding comprises detecting antibody-EphA2 binding.

30. The method of claim 29 wherein the antibody binds to an intracellular epitope of EphA2.

Ans
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31. The method of claim 29 wherein the antibody is produced by hybridoma cell line D7.

32. The method of claim 29 wherein the antibody is labeled with a detectable label, and wherein detecting reagent-EphA2 binding comprises detecting the label.

Ans
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33. The method of claim 32 wherein the antibody comprises at least one of a fluorescent label, a chemiluminescent label, a bioluminescent label, an enzymatic label, a chromogenic label and a radiolabel, wherein detecting reagent-EphA2 binding comprises detecting at least one detectable label.

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34. The method of claim 28 wherein the cell population comprises cells selected from the group consisting of breast cells, kidney cells, prostate cells, lung cells and colon cells.

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35. The method of claim 28 wherein the cell population comprises epithelial cells.

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36. The method of claim 28 wherein the cell population comprises cells selected from the group consisting of breast cancer cells, kidney cancer cells, prostate cancer cells, lung cancer cells and colon cancer cells.

37. The method of claim 28 wherein the cell population comprises epithelial cancer cells.

38. The method of claim 28 wherein the cell population comprises metastatic or potentially metastatic cancer cells.

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39. The method of claim 38 wherein the metastatic or potentially metastatic cancer cells comprise cells selected from the group consisting of breast cancer cells, kidney cancer cells, prostate cancer cells, lung cancer cells, and colon cancer cells.

40. The method of claim 3 wherein the metastatic or potentially metastatic cancer cells comprise epithelial cancer cells.

41. The method of claim 28 wherein the cell population comprises cells from a tissue biopsy.

42. The method of claim 41 wherein the tissue comprises breast tissue or prostate tissue.

43. The method of claim 28 wherein the cell population comprises cells from a body fluid.

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44. The method of claim 43 wherein the body fluid is selected from the group consisting of blood, plasma, spinal fluid, saliva, and urine.

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45. The method of claim 28 wherein detecting reagent-EphA2 binding comprises utilizing a diagnostic method selected from the group consisting of an ELISA assay, a Western blot, and flow cytometry.

46. The method of claim 28 wherein detecting reagent-EphA2 binding comprises utilizing a Western blot; the method further comprising Western blotting with a second antibody having phosphotyrosine specificity.

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47. A method for detecting the presence of metastatic or potentially metastatic cells in a cell population comprising:

incubating at least a portion of the cell population with a reagent capable of binding to EphA2 to allow binding of the reagent to EphA2; and

detecting reagent-EphA2 binding, wherein reagent-EphA2 binding is indicative of the presence of metastatic or potentially metastatic cells in the cell population.

48. The method of claim 47 wherein the reagent comprises an antibody and wherein detecting reagent-EphA2 binding comprises detecting antibody-EphA2 binding.

49. The method of claim 48 wherein the antibody binds to an intracellular epitope of EphA2.

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C12

50. The method of claim 48 wherein the antibody is produced by hybridoma cell line D7.

51. The method of claim 48 wherein the antibody binds to an extracellular epitope of EphA2.

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cont
52. The method of claim of claim 48 wherein antibody-EphA2 binding yields a bound complex comprising a whole cell.

53. The method of claim 52 wherein detecting antibody-EphA2 binding comprises subjecting the bound complex to immunohistochemical staining.

54. The method of claim 48 wherein the antibody is produced by hybridoma cell line B2D6.

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55. The method of claim 48 wherein the bound antibody comprises a detectable label; and wherein detecting antibody-EphA2 binding comprises detecting the label.

56. The method of claim 48 wherein the bound antibody comprises at least one of a fluorescent label, a chemiluminescent label, a bioluminescent label, an enzymatic label, a chromogenic label and a radiolabel; and wherein detecting antibody-EphA2 binding comprises detecting at least one detectable label.

57. The method of claim 47 wherein the cell population comprises cells selected from the group consisting of breast cells, kidney cells, prostate cells, lung cells and colon cells.

58. The method of claim 47 wherein the cell population comprises epithelial cells.

59. The method of claim 47 wherein the cell population comprises cells selected from the group consisting of breast cancer cells, kidney cancer cells, prostate cancer cells, lung cancer cells and colon cancer cells.

60. The method of claim 47 wherein the cell population comprises epithelial cancer cells.

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61. The method of claim 47 wherein the cell population comprises metastatic or potentially metastatic cancer cells.

62. The method of claim 61 wherein the metastatic or potentially metastatic cells comprise cells selected from the group consisting of breast cancer cells, kidney cancer cells, prostate cancer cells, lung cancer cells, and colon cancer cells.

63. The method of claim 47 wherein the metastatic or potentially metastatic cells comprise epithelial cancer cells.

64. The method of claim 47 wherein the cell population comprises cells from a tissue biopsy

65. The method of claim 64 wherein the tissue comprises breast tissue or prostate tissue.

66. The method of claim 47 wherein the cell population comprises cells from a body fluid.

67. The method of claim 66 wherein the body fluid is selected from the group consisting of blood, plasma, spinal fluid, saliva, and urine.

68. The method of claim 47 wherein detecting reagent-EphA2 binding comprises utilizing a diagnostic method selected from the group consisting of an ELISA assay, a Western blot, and flow cytometry.

69. The method of claim 47 wherein detecting reagent-EphA2 binding comprises utilizing a Western blot; the method further comprising Western blotting with a second antibody having phosphotyrosine specificity.

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70. A method for detecting the presence of metastatic or potentially metastatic cells in a cell population comprising:

lysing at least a portion of the cell population;

incubating the lysed cells with a reagent capable of specific binding to nucleic acid comprising RNA or DNA encoding at least a portion of an EphA2 protein to allow binding of the reagent to the nucleic acid; and

detecting reagent-nucleic acid binding, wherein reagent-nucleic acid binding is indicative of the presence of metastatic or potentially metastatic cells in the cell population.

71. The method of claim 65 wherein the nucleic acid comprises RNA.

72. A method for detecting the presence of cancer cells in a selected cell population comprising:

assaying at least a portion of the selected cell population for at least one of

a change in EphA2 expression level;

a change in EphA2 localization pattern; and

a change in EphA2 phosphorylation content

as compared to the EphA2 expression level, localization pattern and phosphorylation content in an analogous normal cell population;

wherein the change is indicative of the presence of a cancer cell in the selected cell population.

73. The method of claim 72 wherein a change in EphA2 expression level, localization pattern or phosphorylation content is indicative of the presence of metastatic cancer cells in the cell population.

74. The method of claim 72 wherein a change in EphA2 expression level is indicative of the presence of nonmetastatic cancer cells in the cell population.

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75. The method of claim 72 wherein assaying the cell population comprises incubating at least a portion of the selected cell population with a reagent capable of binding to EphA2 to allow binding of the reagent to EphA2; and detecting reagent-EphA2 binding.

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Cont.

76. The method of claim 75 wherein the reagent is an antibody.

77. The method of claim 76 wherein the antibody is produced by hybridoma D7 or B2D6.

78. A method for determining the disease stage in a cell population comprising cancer cells, the method comprising:

assaying at least a portion of the cell population for at least one of

EphA2 expression level;

EphA2 localization; and

EphA2 phosphorylation content; and

determining the disease stage of the cancer cells.

Ans
C14

79. The method of claim 78 wherein assaying the cell population comprises incubating at least a portion of the cancer cell population with a reagent capable of binding to EphA2 to allow binding of the reagent to EphA2; and detecting reagent-EphA2 binding.

80. The method of claim 79 wherein the reagent is an antibody.

81. The method of claim 80 wherein the antibody is produced by hybridoma D7 or B2D6.

82. Hybridoma cell line D7.

83. Isolated hybridoma cell line D7.